Synthesis, Discovery, and Quantitation of Dihomo-Isofurans: Biomarkers for In Vivo Adrenic Acid Peroxidation.
Aurélien de la Torre, Yiu Yiu Lee, Camille Oger, Per Torp Sangild, Thierry Durand, Jetty Chung-Yung Lee, Jean-Marie Galano

To cite this version:

HAL Id: hal-01058061
https://hal.archives-ouvertes.fr/hal-01058061
Submitted on 3 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Synthesis, Discovery, and Quantitation of Dihomo-Isofurans: Biomarkers for In Vivo Adrenic Acid Peroxidation**

Aurélien de La Torre, Yiu Yiu Lee, Camille Oger, Per Torp Sangild, Thierry Durand, Jetty Chung-Yung Lee,* and Jean-Marie Galano*

Abstract: The growing importance of lipidomics, and the interest of non-enzymatic metabolites of polyunsaturated fatty acids (PUFAs) prompted us to initiate the synthesis of novel dihomo-IsoF compounds. Such metabolites of adrenic acid, the main PUFA in white matter, were synthesized using a divergent approach based on an orthoester cyclization. LC-MS/MS investigation on pig brains showed the potential of this novel biomarker for the first time, as a powerful new tool for brain lipid peroxidation assessment.

Oxygenated metabolites of polyunsaturated fatty acids (PUFAs) are key classes of natural products generated by living organisms. The levels are regulated by the presence of reactive oxygen species (ROS). Among them, isoprostanes (IsoPs), liberated through lipid peroxidation of arachidonic acid (AA), and more recently neuroprostanes (NeuroPs), from docosahexaenoic acid (DHA), are widely studied. IsoPs are used as systemic oxidative stress biomarkers in vivo whereas NeuroPs are specific for neurodegenerative diseases. A novel peroxidation pathway of PUFAs was discovered and it generates tetrahydrofuran (THF) derivatives, that is, isofurans (IsoFs) from AA and neurofurans (NeuroFs) from DHA. These compounds could be highly valuable biomarkers of oxidative stress because they appear to be more abundantly produced than their isoprostane counterparts, and because their formation is dependent upon oxygen tension. We previously showed that F2-dihomo-IsoPs, isoprostane metabolites of adrenic acid (AdA, being the most important PUFA in white matter), and 7- and 17-F2-dihomo-IsoPs are biomarkers for the early detection of Rett syndrome (RTT), and subsequently, oxidative damage of the myelin (Scheme 1). It is therefore reasonable to assume that IsoF metabolites of AdA, namely dihomo-IsoFs, do exist and could be complementary biomarkers of RTT and other neuronal damage. In pursuing this hypothesis, the first synthesis of dihomo-IsoFs is described herein. For the first time, concentrations of dihomo-IsoFs were determined by LC-MS/MS to confirm their presence in vivo, particularly in the brain, and to compare them with those of known biomarkers of oxidative stress, mainly IsoPs, NeuroPs, and F2-dihomo-IsoPs.

The biosynthesis of dihomo-IsoFs can theoretically give 32 possible diastereoisomers, and because two biosynthesis pathways coexist two classes of four families are present, that is, the alkenyl-IsoFs and enediol-IsoFs. Therefore, a total of 256 isomers are potentially generated in vivo.

Three different synthetic strategies have been reported for IsoFs and NeuroFs over the past decade by the groups of Falck, Taber, and Zanoni-Vidari. To identify more distinct and relevant metabolites for biological investigation, we developed a versatile strategy based on a novel framework for both alkenyl- and enediol-dihomo-IsoFs.

Retrosynthetic analysis of alkenyl-dihomo-IsoFs enabled us to identify the THF precursors A (Scheme 2). To develop a common scaffold for both alkenyl- and enediol-isofurans, the polyalcohol derivative C would be a suitable target. Two cyclization processes (5-exo-tet for alkenyl and 5-endo-tet for enediol) could be foreseen with a suitably mono-protected orthoester derivative (B for alkenyl-type) following
the use of Borhan’s stereoselective orthoester cyclization of 1,2,α-triols. The compound C should be available from trans-β-muconic acid (2). Following this analysis we now describe the first synthesis of 10-epi-17(RS)-SC-Δ15-11-dihomo-isoF (1).

The synthesis started with a four-step sequence to access the 1,6-diol 4 in 62% yield and in virtually optically pure form (ee > 99%; Scheme 3). Esterification of 2 with acetyl chloride in an anhydrous iPrOH solution and subsequent

Sharpless asymmetric dihydroxylation gave the corresponding diol 3. Acetonide protection and ester reduction with LiAlH₄ gave 4 on large scale (15 g). This C₂-symmetric structure was then selectively mono-oxidized and elongated by a Wittig reaction in the same pot, thereby resulting in the α,β-unsaturated ester 5 in 62% yield. Protection of the remaining free alcohol yielded the corresponding PMB ether prior to another Sharpless asymmetric dihydroxylation, thus affording the diol 6 in good yield and good diastereoselectivity (d.r. = 88:12).

Regioselective monoprotection of 6 was achieved using Kusumoto’s method through orthoester hydrolysis. Treatment of 6 with trimethyl orthoacetate and PTSA resulted in placing the acyl group in the β-position (4:1). However, subsequent acidic cleavage of the acetonide with PTSA caused a migration of the acyl moiety to the liberated 1,2-diol. Nevertheless, monoprotection was achieved by using trimethyl orthobenzoate to introduce the benzoyl group to the β-position of 7, which was isolated in addition to 8 with a 4:1 regioselectivity (Scheme 4). Interestingly, the benzoyl group did not migrate from the β-position under acidic conditions, but did migrate from the α- to the β-position under basic conditions. Thus, the mixture of regioisomers 7 and 8 was directly treated with PTSA in MeOH/H₂O (9:1), followed by a basic quench with solid NaHCO₃ to afford the triol 9 as a single regioisomer. The triol 9 was then treated under reaction conditions developed by the group of Borhan, that is, orthoester formation with MeCl(OMe)₂ and PPTS, followed by in situ addition of catalytic BF₃·Et₂O to conduct the intramolecular attack at the orthoester intermediate. The cyclization process was extremely temperature dependent. Lewis acid addition at 30°C led to consecutive cleavage of the PMB group, whereas running it at 0°C prevented cyclization and deprotection. Best results were obtained at 15°C, thus yielding the THF derivative 10 in 60% yield. The relative configuration of 10 was confirmed by NOESY NMR. At the stage of the synthesis and after purification by flash chromatography, 10 appears to be free of the minor diastereoisomer observed when 6 was isolated. The Ac and Bz protecting groups of 10 were subsequently transformed into TBS groups by methanalysis with K₂CO₃ in MeOH followed by addition of TBSCI. Reduction of the methyl ester group with LiBH₄.
gave the compound 11 in 62% yield over three steps. This compound is the key intermediate for the generation of all alkyl-IsoFs, alkyl-dihomo-IsoFs, and alkyl-NeuroFs.

The primary alcohol 11 was transformed into the corresponding aldehyde with the Dess–Martin periodinane and followed by a Horner–Wadsworth–Emmons reaction with the commercially available phosphonate 12 (Scheme 5). The cleavage of the PMB group provided the primary alcohol under Luche/C29s conditions and protected with a TBS group.

\[ \text{RS}^{+} = \text{NaHMDS, THF, RT, 60\% over two steps; e) DDQ, CH}_2\text{Cl}_2/H_2\text{O (10:1), 0\°C to RT, 60\% over two steps; f) DMP, CH}_2\text{Cl}_2, RT; g) 15, NaHMDS, THF, \sim -78\°C to RT, 65\% over two steps; h) TBAF, THF, RT; i) LiOH, THF/H_2\text{O (1:1), RT, 76\% over two steps. DMP = Dess–Martin periodinane, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, NaHMDS = sodium bis[trimethylsilyl]amide, TBAF = tetrabutylammonium fluoride.} \]

Scheme 5. Reagents and conditions: a) DMP, CH_2Cl_2, RT; b) Ba(OH)_2, THF, RT, 60% over two steps; c) CeCl_3.7H_2O, NaBH_4, MeOH, 0°C; d) TBSCl, imidazole, DMAP, CH_2Cl_2, RT, 85% over two steps; e) DDQ, CH_2Cl_2/H_2O (10:1), 0°C to RT, 80%; f) DMP, CH_2Cl_2, RT; g) 15, NaHMDS, THF, \sim -78°C to RT, 65% over two steps; h) TBAF, THF, RT; i) LiOH, THF/H_2O (1:1), RT, 76% over two steps. DMP = Dess–Martin periodinane, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, NaHMDS = sodium bis[trimethylsilyl]amide, TBAF = tetrabutylammonium fluoride.

ene 13 was obtained in 60% yield and thus further reduced under Luche’s conditions and protected with a TBS group. Cleavage of the PMB group provided the primary alcohol 14 in 68% yield over three steps. Another oxidation/Wittig sequence with the phosphonium bromide 15 gave the ethyl ester 16 in 65% yield. Finally, exhaustive silyl ether deprotection and saponification of the ester group yielded 10-epi-17(RS)-SC-Δ^11-11-dihomo-IsoF (1) in 76% yield.

Brain tissue consumes approximately 20% of the total oxygen in the body. This consumption increases the likelihood of brain lipid peroxidation when injured because of the high abundance of PUFA, especially AA, AdA, and DHA. The localization of PUFA in the brain is specific: DHA is enriched in grey matter and AdA is found in white matter, whereas AA and EPA are evenly distributed. These brain PUFA are subject to lipid peroxidation (LPO) and oxidative damage within the central nervous system. Furthermore, the products released upon LPO may depend on the oxygen tension in the brain: a higher tension was reported in brain white matter compared to grey matter.[6,19] The difference in PUFA localization and oxygen tension suggests the importance of measuring the appropriate LPO metabolites in different parts of the brain. Lipids of the prefrontal cortex and medial prefrontal cortex of preterm pig (116 days) brains were extracted by Folch solution and subjected to alkaline hydrolysis. Thereafter, AA, DHA, and AdA and the LPO metabolites were purified by anionic solid-phase extraction and analyzed by LC-MS/MS.[20] It was found that the levels of DHA/AA:AdA amounted to approximately 1.5:0.1 in the prefrontal cortex and 1.2:0.07 in the medial prefrontal cortex. These levels represent the total PUFA concentration of the brain tissue without differentiating grey and white matter.

The determination of LPO metabolites revealed significantly high concentrations of 7(RS)-7-F_2-Isop-10-F_4t-NeuroP and 4(RS)-4-F_4t-NeuroP in the prefrontal cortex compared to 15-F_2-Isop, 10-F_4t-NeuroP, or 17(RS)-17-F_2-dihomo-IsoP (Figure 1A). Similarly, the levels of 4(RS)-4-F_4t-NeuroP and 7(RS)-7-F_2-dihomo-IsoP were significantly higher in the medial prefrontal cortex compared to those of 5-F_2-IsoP and 10-F_4t-NeuroP (Figure 1B).

Interestingly, despite the relatively low concentration of AdA, 10-epi-17(RS)-SC-Δ^11-11-dihomo-IsoF levels in both tissues were three- to fourfold higher than those of IsoFs and NeuroFs (Figures 1C and D). This difference is significant, since only up to 32 stereoisomers (2^5) of the particular regioisomeric dihomo-IsoF series, represented by the standard 1, out of the 256 theoretical isomers were specifically measured, whereas IsoFs and NeuroFs levels represent the sum of all 256 and 512 potential metabolites, respectively.

The growth and development of the pig brain is similar to that of the human brain, therefore the results have a significant impact on the study of neuronal damage in humans. Primarily, our study revealed that 10-epi-17(RS)-SC-Δ^11-11-dihomo-IsoF levels were extraordinarily high compared to IsoFs and NeuroFs in the prefrontal cortex tissue of pig brains. Secondly, the dihomo-IsoF levels are comparable to those of 4(RS)-4-F_4t-NeuroP (Figure 1A vs C), which is to date the most prominent biomarker for oxidative stress related neuronal damage.[5] These results show that the whole spectrum of isoprostanoïd and isofuranoid metabolites represented by the novel 10-epi-17(RS)-SC-Δ^11-11-dihomo-IsoF from AdA and known 4(RS)-4-F_4t-NeuroP from DHA should be considered when evaluating neuronal damage and disease. Moreover, the isofuranoid AdA metabolite 1 has a much higher thermal and oxidative stability than 4(RS)-4-F_4t-NeuroP, and should lead to significantly more reliable and reproducible determination of LPO in brain tissues.

Importantly, the pig brain samples of the present study were at a homeostatic state. This state delineates the present results from those of a previous report,[21] which found elevated IsoF levels at greater than 21% oxygen tension and elevated NeuroF at greater than 40% in the prefrontal cortex of piglets after resuscitation from hypoxiaemia compared to nontreated controls.

A damaged prefrontal cortex is associated with cognitive dysfunction, bipolar disorder, and schizophrenia patients.[22,23] This association parallels the recent finding of a relationship to oxidative stress, because the myelin fraction in neuronal
cells from the prefrontal cortex of bipolar disorder patients had increased F2-IsoPs levels compared to controls. In conclusion, a flexible strategy allowed the total synthesis of both C17 epimers of 10-epi-SC-Δ^{15,11}-dihomo-IsoF in 22 steps with a 1.5% overall yield. 10-epi-SC-Δ^{15,11}-Dihomo-IsoF was discovered as a major metabolite of nonenzymatic free-radical-mediated peroxidation of AdA in the prefrontal and medial cortex of pig brain at homeostatic state. Thus, the new isofuran metabolite 10-epi-17(RS)-SC-Δ^{15,11}-dihomo-IsoF from AdA and known 4(RS)-4-F_4t-NeuroP from DHA should be considered as the leading complementary LPO biomarkers when evaluating neuronal damage and disease, whereby the new isofuran metabolite will have the advantage of considerably higher stability compared to NeuroP. Their quantification in the brain will likely provide beneficial insights into neurochemistry and shed light on intervention studies of neurobiology.

**Keywords:** biomarkers · lipids · mass spectrometry · oxidation · total synthesis